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Structure–activity relationships of epolactaene analogs as DNA polymerases inhibitors

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Abstract—Epolactaene, a neuritogenic compound in human neuroblastoma cells, showed inhibitory activities against DNA polymerases α and β. The synthesis and inhibitory activities of epolactaene analogs are described. The α,β-epoxy-γ-lactam moiety in the core and the length of the side chain greatly influenced the activities. Compound **5** was the strongest inhibitor of DNA polymerase α and β of all synthesized compounds with IC_{50} values of 13 and 78 μM, respectively. *N*- and *O*-alkyl derivatives that had modified core moieties showed moderate inhibition.

1. Introduction

Epolactaene (**1**) was isolated from the culture supernatant of *Penicillium* sp. BM 1689-P by Kakeya et al. in 1995 (Fig. 1).[1] It induced neurite outgrowth and arrested the cell cycle at the G1 phase in a human neuroblastoma cell line SH-SY5Y. The characteristic features of epolactaene include the α,β-epoxy-γ-lactam moiety in the core and the conjugated triene moiety in the side chain. The significant biological activity as well as the structural complexity of epolactaene has stimulated intensive synthetic interest. In 1998, Hayashi's group and Kogen's group, respectively, accomplished the total synthesis of **1** and determined its absolute stereochemistry.[2] We also accomplished the total synthesis of epolactaene based on the bridgehead oxiranyl anion strategy in 1999.[3]

Biological studies of epolactaene and analogs have been under way. Kakeya et al. found that MT-5, 3-acetyl-4,5-dimethyl-5-octadecyloxy-3-pyrrolin-2-one possessed neuritogenic activity in SH-SY5Y cells and also arrested cell cycle progression at G0/G1 like epolactaene (Fig. 2).[4] And they found that 3-acetyl-4,5-dimethyl-5-hydroxy-*N*-alkyl-3-pyrrolin-2-ones with a straight long alkyl group, for example, MT-19, MT-20, and MT-21, showed neuritogenic effect in rat pheochromocytoma PC12 cells. They reported later that MT-21 induced

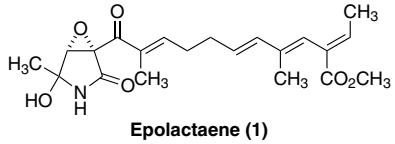

Figure 1. Structure of epolactaene (**1**).

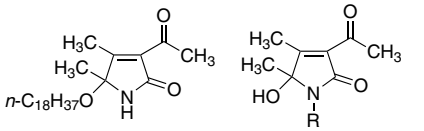

Figure 2. Structure of MT-5, MT-19, MT-20, and MT-21.

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apoptosis via the direct release of cytochrome *c* from the mitochondria.[4b,c]

We also prepared several analogs of epolactaene (**2**–**4**) and carried out biological studies (Fig. 3).[3c] Then we found that epolactaene **1** and its analog **2** selectively inhibited the activities of mammalian DNA polymerases α (pol α), β (pol β), and human DNA topoisomerase II (topo II).[5] The synthetic anologs **3** and **4** did not affect pol α and pol β at concentrations lower than 200 μM. We also reported that synthetic analogs (**1**–**4**) induced apoptosis in BALL-1 cells and that the α,β-epoxy-γ-lactam in the core and the straight long alkyl group in the side chain were very important for the activities.[6]

We wish to report the structure–activity relationships of newly synthesized epolactaene analogs focusing on the inhibitory activities against DNA polymerase α and β.

2. Chemistry

We have already reported the preparation of **1**–**5**, which was outlined in Scheme 1.[3c] The TBAF-catalyzed reaction of the silylated epoxylactone **6** and aldehydes **7**, followed by desilylation of the OTMS group with aq HF gave the aldol-type products **8**. After oxidation of **8**, the resulting ketone **9** was converted into the hydroxyamide **10** by ammonolysis. Oxidation of **10** gave the α,β-epoxy-γ-lactams (**1**–**5**).

As we focused on the α,β-epoxy-γ-lactam moiety, the synthetic analogs (**11**–**19**) were also prepared (Fig. 4).

Scheme 2 outlines the synthesis of **11** and **12**. Treatment of **9b** with 1-dodecylamine in THF, followed by oxidation with TPAP and NMO in the presence of MS4A gave **11**.[7] And the treatment of **2** with 10-camphorsulfonic acid in MeOH gave **12** in 80% yield. The stereochemistry of **12** was determined by the NOESY spectrum. Thus, NOESY correlation for OCH_3/H-3 was observed.

The preparation of *N*-alkyl-α,β-epoxy-γ-lactams (**13**–**14**) and *N*-alkyl-α, β-epoxy-γ-hydroxyamides (**15**–**16**) is shown in Scheme 3. Treatment of **20** with 1-alkylamine in THF gave hydroxyamides (**15**–**16**). And oxidation of hydroxyamides with TPAP and NMO gave *N*-alkyl-α,β-epoxy-γ-lactams (**13**–**14**) as a tautomeric mixture. Judging from the ^1H NMR spectra, we observed that the tautomeric ratio of *N*-alkyl-α,β-epoxy-γ-lactams in CD_3OD differed from that in $CDCl_3$ (Table 1). Compounds **13** and **14** existed mainly in the γ-lactam in CD_3OD. On the other hand, **13** and **14** existed in a tautomeric mixture of the γ-lactam (**a**+**b**) and keto-amide (**c**) in $CDCl_3$.

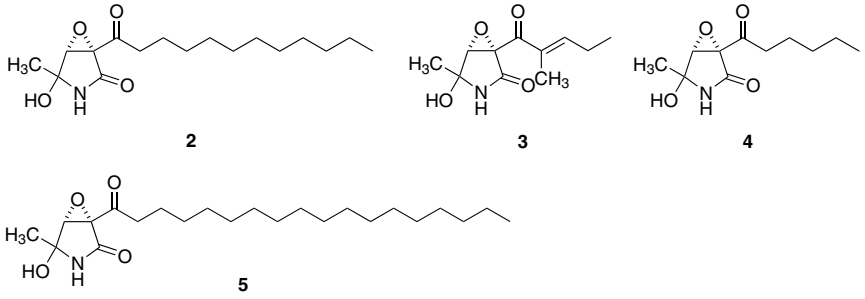
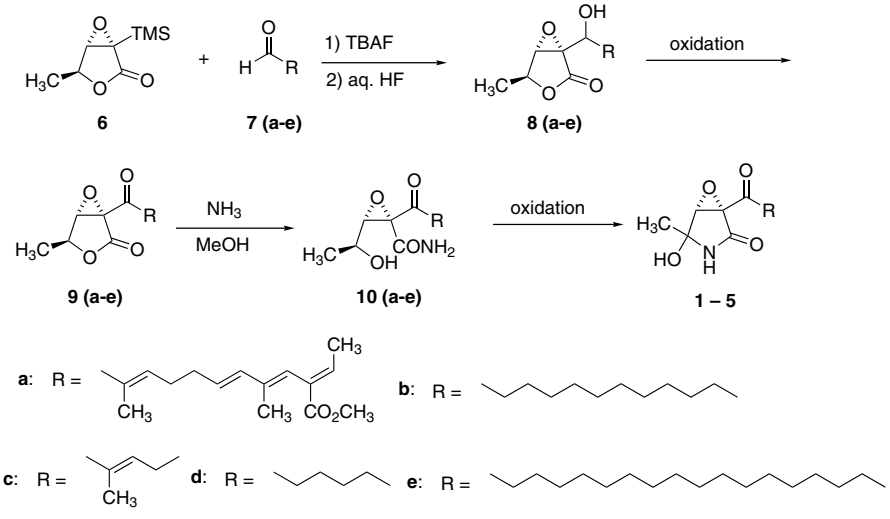


Figure 3. Structure of epolactaene analogs (**2**–**4**).



Scheme 1. Synthesis of epolactaene (**1**) and its analogs (**2**–**5**).

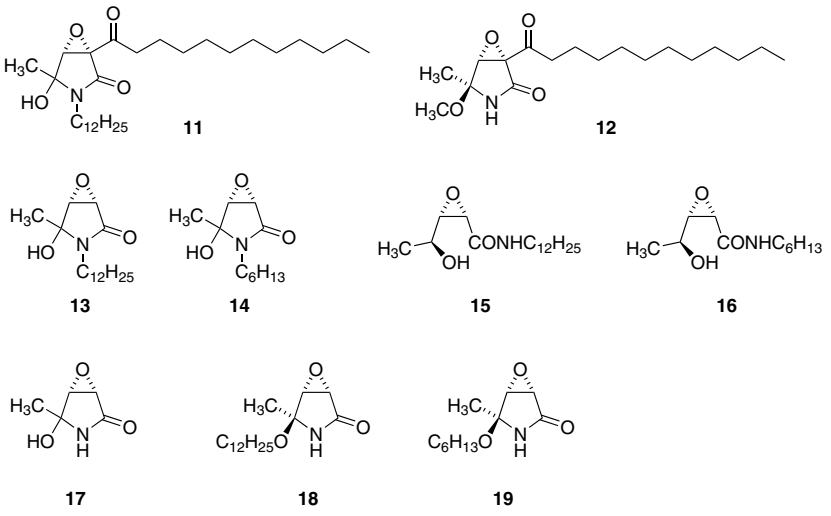
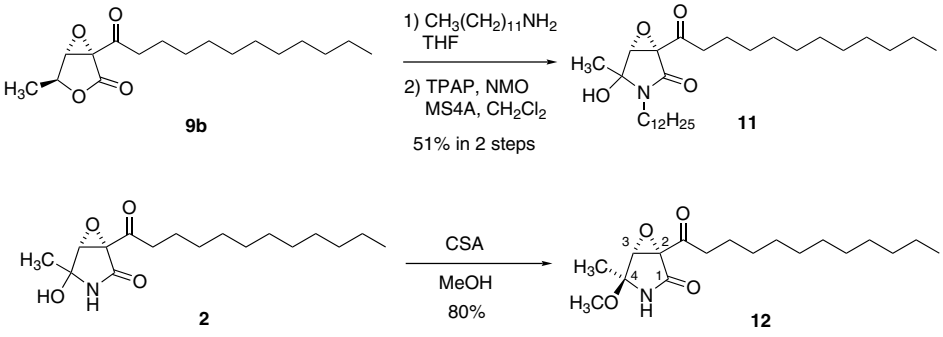
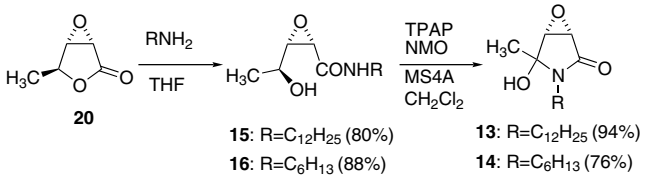


Figure 4. Structure of newly synthesized epolactaene analogs.

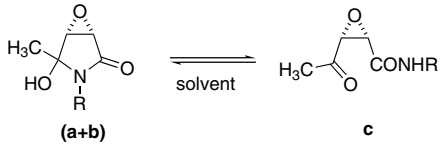


Scheme 2. Synthesis of **11** and **12**.



Scheme 3. Synthesis of *N*-alkyl derivatives (**13** and **14**).

Table 1. Tautomeric ratio of **13** and **14** in CD_3OD and $CDCl_3$, measured by ^1H NMR (400 MHz)

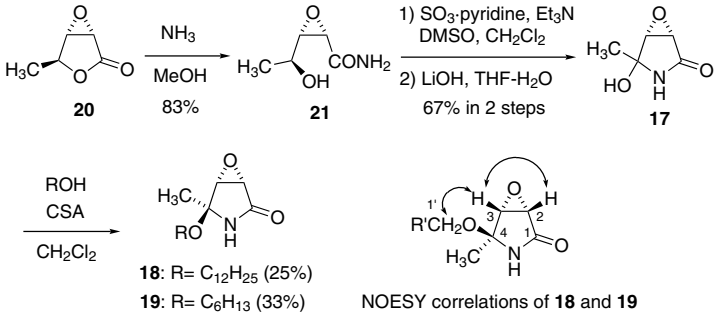


R	Solvent	**a**+**b**:**c**
$C_{12}H_{25}$ (**13**)	CD_3OD	22:1
$C_{12}H_{25}$ (**13**)	$CDCl_3$	1:1.5
C_6H_{13} (**14**)	CD_3OD	12:1
C_6H_{13} (**14**)	$CDCl_3$	1:1.8

The synthesis of γ-alkoxy-α,β-epoxy-γ-lactams (**18**–**19**) is shown in Scheme 4. After ammonolysis of **20**, the resulting hydroxyamide **21** was oxidized by Swern oxidation. Since a mixture of **17** and O-trifluoroacetylated **17** was obtained, the crude mixture was treated with LiOH in THF–H_2O to give **17**. And treatment of **17** with alcohols under acidic conditions gave γ-alkoxy-α,β-epoxy-γ-lactams (**18**–**19**). The stereochemistry of **18** and **19** was determined by NOE experiments (NOEs between H-3/H-1′).

3. DNA polymerase inhibitory activity

The inhibitory activities of **1**–**5**, **9**(**a**–**d**), **10**(**a**–**d**), and **11**–**19** against pols α and β are summarized in Table 2. Compounds **1**, **2**, **5**, **11**, **12**, and **18** inhibited the activities of pol α and pol β. The inhibition by each of the compounds was dose dependent. The inhibitory effect on pol α, which is a replicative DNA polymerase, by these compounds was stronger than that on pol β which is a repair-related DNA polymerase. We found that epolactaene did not affect the activities of higher plant DNA polymerase I and II, prokaryotic DNA polymerases,



Scheme 4. Synthesis of *O*-alkyl derivatives (**18** and **19**).

Table 2. IC_{50} values of enzymatic inhibition against mammalian DNA polymerases α (pol α) and β (pol β)

Compounds	IC_{50} (μM)	
	Pol α	Pol β
1	25	94
2	26	98
3	>200	>200
4	>200	>200
5	13	78
9 (a–d)	>200	>200
10 (a–d)	>200	>200
11	27	99
12	33	98
13	81	>200
14	>200	>200
15	>200	>200
16	>200	>200
17	>200	>200
18	27	95
19	>200	>200

HIV-1 reverse transcriptase, calf thymus terminal deoxynucleotidyl transferase, and DNA metabolic enzymes.[5] Compound **5** was the strongest inhibitor of all synthesized compounds. Since the molecular length of **5** was the largest of all synthesized compounds, and since the compounds which were the length of more than 11 Å inhibited the activities of pols α and β (Table 3), the length of the alkyl side chain, that is the hydrophobicity, greatly affected the inhibitory activities. These results were also clarified by the calculated log*P* values (partition coefficients for octanol/water).[6]

On the other hand, it is very interesting that lactones **9(a–d)** and hydroxyamides **10(a–d)** had no influence on pol α and pol β at concentrations lower than 200 μM. So the α, β-epoxy-γ-lactam in the core was important for the activities.

As the inhibitory activities of **11** and **12** were very similar to those of **2**, the *N*- and *O*-alkyl groups did not influence the activities of **2**.

Since *N*-dodecyl derivative **13** and *O*-dodecyl derivative **18** had the inhibitory activities, the acyl group at the 3-position was not necessarily required for the inhibition of pol activities. Although **13** showed weak activity, **18** had almost equal inhibitory activities to **2**. The activities

Table 3. The molecular length and log*P* value of the synthesized compounds calculated by three-dimensional computer simulation

Compounds	Molecular length (Å)	Log*P*
1	18.448	1.85
2	18.445	2.80
3	10.733	0.20
4	10.807	0.29
5	25.991	5.30
11	17.774	7.61
12	19.686	3.16
13	17.123	3.69
14	9.465	1.18
17	3.612	−1.13
18	18.723	3.81
19	10.723	1.31

These data were obtained using CS Chem-3D version 6.0 software (Cambridge Soft, USA). A restrained molecular dynamics method was used to calculate the energetically minimum structures of compounds.

were significantly affected by the length of the alkyl group in case of both *N*-alkyl derivatives (**13** and **14**) and *O*-alkyl derivatives (**18** and **19**).

N-alkyl hydroxyl amides (**15** and **16**) had no influence on pol α and pol β at concentrations lower than 200 μM. Compound **17**, which has no hydrophobic moiety at the α,β-epoxy-γ-lactam did not inhibit the activities of the pols. So, it appears that the α,β-epoxy-γ-lactam as well as the hydrophobicity would be very important for the inhibitory activities.

4. Conclusion

We have synthesized epolactaene analogs and evaluated their inhibitory activity against pols. Both the α,β-epoxy-γ-lactam in the core and the length of the alkyl side chain were very important for the activities. Thus, compounds **1**,**2**, **5**, **11**, **12**, and **18** inhibited the activities of pol α and pol β. Compound **5** possessing the longest alkyl side chain showed the most potent activity among the synthesized analogs.

5. Experimental

5.1. General

^{1}H and ^{13}C NMR were recorded on a JEOL JNM-400 or on a BRUKER DXR400 or DRX600. Chemical

shifts were reported in δ, parts per million (ppm), relative to TMS as an internal standard or calibrated using residual undeuteriated solvent as an internal reference. IR spectra were recorded on a JASCO FT/IR-410 spectrometer. Mass spectra were obtained on API QSTAR Pulsar i spectrometer. Optical rotations were measured on a JASCO P-1030 digital polarimeter. Melting points were determined with Yanaco MP-3S melting point apparatus. Column chromatography was carried out on Fuji Silisia PSQ100B. Analytical thin-layer chromatography (TLC) was performed on pre-coated Merck silica gel 60 F_{254} plates, and compounds were visualized by UV illumination (254 nm) or heating 150 °C after spraying phosphomolybdic acid in ethanol. THF was distilled from sodium/benzophenone. CH_2Cl_2 was distilled from P_2O_5. DMSO was distilled from CaH_2. All other solvent and reagents were obtained from commercial sources and used without further purification. Organic extracts were dried over $MgSO_4$, filtered, concentrated using a rotary evaporator. Involatile oils and solids were vacuum dried.

5.1.1. (1*R*,5*R*)-1-Dodecanoyl-3-dodecyl-4-hydroxy-4-methyl-6-oxa-3-aza-bicyclo[3.1.0]hexan-2-one (11). A solution of **9b** (28 mg, 0.095 mmol) and 1-dodecylamine (21 mg, 0.11 mmol) in THF (3 mL) was stirred at rt for 17 h. Then the mixture was evaporated. To a stirred solution of the residue, NMO (15 mg, 0.13 mmol) and MS4A (0.15 g) in CH_2Cl_2 (3 mL) was added TPAP (2.0 mg, 5.7 μmol) at rt and the mixture was stirred at rt for 1.5 h. The mixture was directly purified by column chromatography with a 5:1 mixture of hexane and EtOAc as eluents to give **11** (23 mg, 0.048 mmol, 51% in two steps) as a ca. 3.2:1 tautomeric mixture, as a colorless oil. $[\alpha]_D^{24}$ −63.6 (*c* 0.54, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, a major tautomer): δ 0.88 (6H, t, $J = 6.8$ Hz), 1.24–1.30 (34H, br m), 1.43–1.48 (2H, m), 1.55 (3H, s), 1.59–1.63 (2H, m), 2.35–2.40 (1H, m), 2.48–2.52 (1H, m), 3.13–3.25 (2H, m), 4.11 (1H. s), 4.40 (1H, br s); ^{13}C NMR (100 MHz, $CDCl_3$, both tautomers): δ 14.1, 20.4, 22.5, 22.7, 27.2, 28.6, 28.9, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 38.9, 39.3, 60.9, 63.1, 65.0, 86.2, 166.3, 202.3; IR (neat) cm^{-1}: 3384, 2925, 2854, 1731, 1685, 1466, 1419, 1375, 1325, 1215, 1155, 954, 910, 760, 666; HRMS, calcd for $C_{29}H_{53}NO_4Na$ $(M+Na)^+$ 502.3872, found 502.3863.

5.1.2. (1*R*,4*R*,5*R*)-1-Dodecanoyl-4-methoxy-4-methyl-6-oxa-3-aza- bicyclo[3.1.0]hexan-2-one (12). A solution of **2** (21 mg, 0.067 mmol) and 10-camphorsulfonic acid (3.8 mg, 0.016 mmol) in MeOH (3 mL) were stirred at rt for 1.5 h. After being stirred at 50 °C for 4.5 h, the reaction mixture was diluted with EtOAc and washed with H_2O and brine. The organic layer was dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography with a 2:1 mixture of hexane and EtOAc as eluents to give **12** (17 mg, 0.053 mmol, 80%) as a colorless oil. $[\alpha]_D^{24}$ −93.4 (*c* 0.39, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.88 (3H, t, $J = 7.0$ Hz), 1.25–1.32 (16H, br m), 1.54 (3H, s), 1.58–1.64 (2H, m), 2.59 (2H, t, $J = 7.2$ Hz), 3.28 (3H, s), 3.90 (1H, br s), 6.08 (1H, br s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 20.6, 22.6, 22.7, 29.0, 29.3, 29.4, 29.6, 29.6, 31.9, 39.9, 49.8, 61.3, 63.8, 86.8, 168.6, 199.8; IR (neat) cm^{-1}: 3284, 2925, 2854, 1737, 1465, 1404, 1378, 1340, 1174, 1123, 1056, 902, 875, 761, 655; HRMS, calcd for $C_{18}H_{31}NO_4Na$ $(M+Na)^+$ 348.2150, found 348.2131.

5.1.3. (3*S*,4*S*)-3-{(1′*S*)-1′-Hydroxyethyl}oxirane-2-carboxylic acid dodecylamide (15). A solution of **20** (101 mg, 0.89 mmol) and 1-dodecylamine (174 mg, 0.94 mmol) in THF (5 mL) was stirred at rt for 2 h. Then the mixture was evaporated. The residue was purified by column chromatography with a 1:1 mixture of hexane and EtOAc as eluents to give **15** (214 mg, 0.71 mmol, 80%) as a white solid. Mp=65–66 °C; $[\alpha]_D^{24}$ −3.2 (*c* 1.1, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (3H, t, $J = 6.9$ Hz), 1.26–1.30 (18H, br m), 1.37 (3H, d, $J = 6.3$ Hz), 1.53 (2H, t, $J = 7.0$ Hz), 2.88 (1H, br s), 3.08 (1H, dd, $J = 8.2$ Hz, 4.6 Hz), 3.28 (2H, q, $J = 7.0$ Hz), 3.49 (1H, qd, $J = 6.3$ Hz, 8.2 Hz), 3.57 (1H, d, $J = 4.6$ Hz), 6.21 (1H, br s);^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 20.3, 22.7, 26.9, 29.2, 29.3, 29.4, 29.5, 29.5, 29.6, 29.6, 31.9, 39.1, 55.2, 60.9, 65.3, 167.2; IR (KBr) cm^{-1}: 3296, 2959, 2919, 2848, 1655, 1559, 1468, 1373, 1320, 1281, 1158, 1076, 955, 892, 843, 820, 722, 652; HRMS, calcd for $C_{17}H_{33}NO_3Na$ $(M+Na)^+$ 322.2358, found 322.2397.

5.1.4. (1*S*,5*R*)-3-Dodecyl-4-hydroxy-4-methyl-6-oxa-3-aza-bicyclo[3.1.0]hexan-2-one (13). To a stirred solution of **20** (30 mg, 0.10 mmol), NMO (19 mmol, 0.16 mmol), and MS4A (0.1 g) in CH_2Cl_2 (2 mL) was added TPAP (3.5 mg, 10 μmol) at rt and the mixture was stirred at rt for 1 h. The mixture was directly purified by column chromatography with a 2:1 mixture of hexane and EtOAc as eluents to give **13** (24 mg, 0.095 mmol, 94%) as a tautomeric mixture, as a colorless oil. $[\alpha]_D^{24}$ −6.4 (*c* 0.55, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD, **13a**:**13b**:**13c**=ca. 17:5:1) **13a**: δ 0.89 (3H, t, $J = 7.4$ Hz), 1.28 (18H, br m), 1.47 (3H, s), 1.53–1.62 (2H, m), 3.04–3.20 (2H, m), 3.64 (1H, d, $J = 2.8$ Hz), 3.80 (1H, d, $J = 2.8$ Hz); **13b**: δ 0.89 (3H, t, $J = 7.4$ Hz), 1.28 (18H, br m), 1.47 (3H, s), 1.53–1.62 (2H, m), 3.04–3.20 (2H, m), 3.63 (1H, d, $J = 2.9$ Hz), 3.85 (1H, d, $J = 2.9$ Hz); **13c**: δ 0.89 (3H, t, $J = 7.4$ Hz), 1.28 (18H, br m), 1.53–1.62 (2H, m), 2.24 (3H, s), 3.04–3.20 (2H, m), 3.70 (1H, d, $J = 5.2$ Hz), 3.84 (1H, d, $J = 5.2$ Hz); ^{1}H NMR (400 MHz, $CDCl_3$, **13a**+**13b**:**13c**= ca. 1:trace:1.5) **13a**: δ 0.88 (3H, t, $J = 6.8$ Hz), 1.26 (19H, br m), 1.44–1.48 (2H, m), 1.56 (3H, s), 1.79 (1H, br s), 3.11 (2H, m), 3.62 (1H, d, $J = 2.8$ Hz), 3.79 (1H, d, $J = 2.8$ Hz); **13c**: δ 0.88 (3H, t, $J = 6.8$ Hz), 1.26 (19H, br m), 1.44–1.48 (2H, m), 2.29 (3H, s), 3.19 (2H, m), 3.74 (1H, d, $J = 5.3$ Hz), 3.87 (1H, d, $J = 5.3$ Hz), 6.37 (1H, br s); ^{13}C NMR (100 MHz, CD_3OD, **13a**+**13b**): δ 14.5, 20.9, 23.8, 28.1, 28.3, 30.0, 30.4, 30.5, 30.7, 30.8, 33.1, 39.8, 39.9, 52.6, 53.4, 57.4, 59.3, 88.0, 88.4, 172.4; ^{13}C NMR (100 MHz, $CDCl_3$, **13a**+**13c**): δ 14.1, 20.5, 22.7, 26.7, 27.1, 28.0, 28.9, 29.2, 29.3, 29.5, 29.5, 29.5, 29.6, 31.9, 38.8, 39.1, 51.5, 55.4, 58.0, 58.8, 87.3, 164.4, 199.9; IR (neat) cm^{-1}: 3351, 3015, 2925, 2854, 1687, 1544, 1466, 1423, 1369,

1215, 1154, 951, 867, 837, 758, 667, 617; HRMS, calcd for $C_{17}H_{31}NO_3Na$ $(M+Na)^+$ 320.2201, found 322.2191.

5.1.5. (3*S*,4*S*)-3-{(1′*S*)-1′-Hydroxyethyl}oxirane-2-carboxylic acid hexylamide (16). A solution of **20** (94 mg, 0.82 mmol) and 1-hexylamine (150 μmL, 1.1 mmol) in THF (3 mL) was stirred at rt for 1 h. Then the mixture was evaporated. The residue was purified by column chromatography with a 1:1 mixture of hexane and EtOAc as eluents to give **16** (156 mg, 0.72 mmol, 88%) as a colorless oil. $[\alpha]_D^{24}$ −6.0 (*c* 3.3, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (3H, t, $J = 6.5$ Hz), 1.30–1.35 (6H, br m), 1.36 (3H, d, $J = 6.3$ Hz), 1.51 (2H, t, $J = 6.9$ Hz), 3.08 (1H, dd, $J = 8.2$ Hz, 4.6 Hz), 3.27 (2H, q, $J = 7.1$ Hz) 3.50 (1H, qd, $J = 6.9$ Hz, 8.2 Hz), 3.57 (1H, d, $J = 4.6$ Hz), 3.62 (1H, br s), 6.34 (1H, br s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.9, 20.3, 22.4, 26.5, 29.3, 29.3, 31.3, 39.1, 55.3, 61.0, 64.8, 167.2; IR (neat) cm^{-1}: 3328, 2958, 2931, 2859, 1660, 1542, 1466, 1372, 1275, 1163, 1216, 1128, 1072, 1038, 958, 895, 829, 757, 667, 611; HRMS, calcd for $C_{11}H_{21}NO_3Na$ $(M+Na)^+$ 238.1419, found 238.1426.

5.1.6. (1*S*,5*R*)-3-Hexyl-4-hydroxy-4-methyl-6-oxa-3-aza-bicyclo[3.1.0]hexan-2-one (14). To a stirred solution of 16 (35 mg, 0.16 mmol), NMO (31 mmol, 0.27 mmol), and MS4A (0.1 g) in CH_2Cl_2 (3 mL) was added TPAP (6.0 mg, 17 μmol) at rt and the mixture was stirred at rt for 1 h. The mixture was directly purified by column chromatography with a 2:1 mixture of hexane and EtOAc as eluents to give **14** (27 mg, 0.12 mmol, 76%) as a tautomeric mixture, as a colorless oil. $[\alpha]_D^{24}$ −28.4 (*c* 0.53, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD, **14a**:**14b**:**14c**=ca. 10:2:1) **14a**: δ 0.90 (3H, t, $J = 7.2$ Hz), 1.29 (6H, br m), 1.47 (3H, s), 1.49–1.62 (2H, m), 3.05–3.24 (2H, m), 3.64 (1H, d, $J = 2.8$ Hz), 3.79 (1H, d, $J = 2.8$ Hz); **14b**: δ 0.90 (3H, t, $J = 7.2$ Hz), 1.29 (6H, br m), 1.47 (3H, s), 1.49–1.62 (2H, m), 3.05–3.24 (2H, m), 3.64 (1H, d, $J = 2.8$ Hz), 3.85 (1H, d, $J = 2.8$ Hz); **14c**: δ 0.90 (3H, t, $J = 7.2$ Hz), 1.29 (6H, br m), 1.44 (2H, m), 2.23 (3H, s), 3.05–3.24 (2H, m), 3.70 (1H, d, $J = 5.2$ Hz), 3.84 (1H, d, $J = 5.2$ Hz); ^{1}H NMR (400 MHz, $CDCl_3$, **14a**:**14b**:**14c**=ca. 1:trace:1.8) **14a**: δ 0.88 (3H, t, $J = 6.8$ Hz), 1.26 (6H, br m), 1.35–1.45 (2H, m), 1.55 (3H, s), 3.12 (2H, m), 3.60 (1H, d, $J = 2.8$ Hz), 3.80 (1H, d, $J = 2.8$ Hz); **14c**: δ 0.88 (3H, t, $J = 6.8$ Hz), 1.26 (6H, br m), 1.35–1.45 (2H, m), 1.53 (3H, s), 3.18 (2H, m), 3.75 (1H, d, $J = 5.3$ Hz), 3.88 (1H, d, $J = 5.3$ Hz); ^{13}C NMR (100 MHz, CD_3OD, **14a**): δ14.4, 20.9, 22.9, 27.1, 29.2, 31.8, 40.6, 51.9, 59.9, 87.9, 170.6; ^{13}C NMR (100 MHz, $CDCl_3$, **14a**+**14c**): δ 14.0, 20.5, 23.2, 27.2, 27.4, 29.5, 29.9, 32.2, 39.4, 39.8, 52.2, 55.6, 59.0, 59.1, 88.0, 167.2, 203.2; IR (neat) cm^{-1}: 3351, 2956, 2931, 2858, 1690, 1550, 1459, 1423, 1373, 1213, 1155, 950, 868, 836, 771, 654, 617; HRMS, calcd for $C_{11}H_{19}NO_3Na$ $(M+Na)^+$ 236.1262, found 236.1231.

5.1.7. (3*S*,4*S*)-3-{(1′*S*)-1-Hydroxyethyl)}oxirane-2-carboxylic acid amide (21). A solution of **20** (310 mg, 2.7 mmol) and 0.5 mL of liquid NH_3 in MeOH (1 mL) was stirred at rt for 2 h. Then the mixture was evaporated. The residue was purified by column chromatography with a 15:1 mixture of $CHCl_3$ and MeOH as eluents to give **21** (296 mg, 2.3 mmol, 83%) as a colorless oil. $[\alpha]_D^{23}$ −15.2 (*c* 0.55, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.38 (3H, d, $J = 6.4$ Hz), 3.10 (1H, dd, $J = 4.6$ Hz, 8.0 Hz), 3.21 (1H, br s), 3.56 (1H, d, $J = 4.6$ Hz), 3.65 (1H, m), 6.29 (2H, br s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.4, 54.8, 60.9, 64.9, 170.3; IR (neat) cm^{-1}: 3437, 2979, 2934, 1674, 1450, 1375, 1311, 1162, 1129, 1077, 1043, 961, 892, 849, 801, 651.

5.1.8. (1*S*,5*R*)-4-Hydroxy-4-methyl-6-oxa-3-aza-bicyclo[3.1.0]hexan-2-one (17). To a solution of DMSO (200 μL, 2.8 mmol) in CH_2Cl_2 (3 mL) was added TFAA (260 μL, 1.8 mmol) at −78 °C and the mixture was stirred at −78 °C for 5 min. A solution of **21** (80.9 mg, 0.62 mmol) in CH_2Cl_2–DMSO (15:1, 3 mL) was added to the mixture at −78 °C and the mixture was stirred at −78 °C for 1 h. Then Et_3N (0.9 mL, 6.5 mmol) was added and the mixture was stirred at rt for 10 min. The mixture was quenched by the addition of H_2O and extracted with EtOAc. The extract was washed with brine, dried ($MgSO_4$), and concentrated. To a solution of the residue in THF–H_2O (20:1, 2 mL) was added LiOH (15 mg, 0.63 mmol) at 0 °C. After being stirred at 0 °C for 10 min, the mixture was directly purified by column chromatography with a 9:1 mixture of $CHCl_3$ and MeOH as eluents to give **17** (54 mg, 0.41 mmol, 67%) as a tautomeric mixture, as a colorless oil. ^{1}H NMR (400 MHz, CD_3OD, **17a**:**17b**:**17c**=ca. 6.5:1:1) **17a**: δ 1.45 (3H, s), 3.57 (1H, d, $J = 2.6$ Hz), 3.77 (1H, d, $J = 2.6$ Hz); **17b**: δ 1.49 (3H, s), 3.57 (1H, d, $J = 2.7$ Hz), 3.82 (1H, d, $J = 2.7$ Hz); **17c**: δ 2.45 (3H, s), 3.71 (1H, d, $J = 5.2$ Hz), 3.84 (1H, d, $J = 5.2$ Hz); ^{13}C NMR (150 MHz, CD_3OD, **17a**): δ 22.0, 53.4, 60.0, 85.3, 174.4; IR (neat) 3305, 2927, 1710, 1630, 1432, 1383, 1273, 1185, 1154, 1057, 1013, 978, 940, 884, 850, 832, 756, 643 cm^{-1}; HRMS, calcd for $C_5H_7NO_3Na$ $(M+Na)^+$ 152.0318, found 152.0332.

5.1.9. (1*S*,4*R*,5*S*)-4-Dodecyloxy-4-methyl-6-oxa-3-aza-bicyclo[3.1.0]hexan-2-one (18). A solution of **17** (29 mg, 0.23 mmol), 1-dodecanol (127 mg, 0.68 mmol), and 10-camphorsulfonic acid (11.3 mg, 0.49 mmol) in CH_2Cl_2 (3 mL) were stirred at rt for 1.5 h. The reaction mixture was diluted with EtOAc and washed with H_2O and brine. The organic layer was dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography with a 5:1 mixture of hexane and EtOAc as eluents to give **18** (17 mg, 0.056 mmol, 25%) as a colorless oil. $[\alpha]_D^{24}$ −36.1 (*c* 0.65, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.88 (3H, t, $J = 6.8$ Hz), 1.26–1.31 (20 H, br m), 1.54 (3H, s), 3.43 (2H, m), 3.63 (1H, br s), 3.76 (1H, br s), 6.10 (1H, br m); ^{13}C NMR (150 MHz, $CDCl_3$): δ 14.1, 21.1, 22.7, 26.1, 29.3, 29.4, 29.5, 29.6, 29.6, 29.6, 29.8, 31.9, 52.1, 57.7, 62.2, 87.6, 171.9; IR (neat) 3240, 2925, 2854, 1722, 1464, 1377, 1210, 1135, 1079, 882, 836, 759, 721, 644 cm^{-1}; HRMS, calcd for $C_{17}H_{31}NO_3Na$ $(M+Na)^+$ 320.2196, found 320.2229.

5.1.10. (1*S*,4*R*,5*S*)-4-Hexyloxy-4-methyl-6-oxa-3-aza-bicyclo[3.1.0]hexan-2-one (19). A solution of **17** (13 mg, 0.10 mmol), 1-hexylalcohol (64 μL, 0.51 mmol), and 10-camphorsulfonic acid (2.4 mg, 10 μmol) in CH_2Cl_2 (3 mL) were stirred at rt for 2 h. The reaction mixture was diluted with EtOAc and washed with H_2O and brine. The organic layer was dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography with a 3:1 mixture of hexane and EtOAc as eluents to give **19** (6.7 mg, 0.031 mmol, 31%) as a colorless oil. $[\alpha]_D^{23}$ −47.2 (*c* 0.59, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.89 (3H, t, $J = 6.8$ Hz), 1.25–1.34 (8 H, br m), 1.55 (3H, s), 3.43 (2H, m), 3.64 (1H, br s), 3.77 (1H, br s), 5.68 (1H, br s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0, 21.2, 22.5, 25.7, 29.7, 31.6, 52.1, 57.7, 62.1, 87.6, 171.8; IR (neat) 3231, 3018, 2932, 2860, 1719, 1462, 1423, 1379, 1216, 1179, 1135, 1081, 959, 881, 850, 837, 758, 668 cm^{-1}; HRMS, calcd for $C_{11}H_{19}NO_3Na$ $(M+Na)^+$ 236.1257, found 236.1282.

5.2. DNA polymerase assays

Pol α was purified from calf thymus by immuno-affinity column chromatography as described previously.[8] Recombinant rat pol β was purified from *E. coli* JMpβ5 as described by Date et al.[9] The reaction mixtures for pol α and β were described previously.[10] The substrates of DNA polymerases were used poly(dA)/oligo(dT)$_{12-18}$ and dTTP as template–primer DNA and nucleotide substrate, respectively. The synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) at various concentrations and sonicated for 30 s. Four microliter of sonicated samples were mixed with 16 μL of each enzyme (final 0.05 units) in 50 mM Tris–HCl (pH 7.5) containing 1 mM dithiothreitol, 50% glycerol, and 0.1 mM EDTA, and kept at 0 °C for 10 min. These inhibitor–enzyme mixtures (8 μL) were added to 16 μL of each of the enzyme standard reaction mixtures, and incubation was carried out at 37 °C for 60 min. The activity without the inhibitor was considered 100%, and the remaining activities at each concentration of inhibitor were determined as percentages of this value. One unit of each DNA polymerase activity was defined as the amount of enzyme that catalyzes the incorporation of 1 nmol of deoxyribonucleotide triphosphates (i.e., dTTP) into synthetic template–primers (i.e., poly(dA)/oligo(dT)$_{12-18}$, A/T=2/1) in 60 min at 37 °C under the normal reaction conditions for each enzyme.[10]

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